IN VIVO TISSUE DISPOSITION OF 3'-AZIDO-3'-DEOXYTHYMIDINE AND ITS ANABOLITES IN CONTROL AND RETROVIRUS-INFECTED MICE

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ABSTRACT:

At present, 3'-azido-3'-deoxythymidine (AZT; zidovudine) remains the drug of choice for initiating AIDS therapy. This drug in itself is inactive; it needs to be converted intracellularly by a series of cellular kinases to AZT 5'-triphosphate (AZT-TP) to exert antiviral activity. The purpose of this study was to examine the in vivo disposition of the phosphorylated AZT anabolites in different target tissues and to investigate the effects of chronic retrovirus infection on the tissue disposition of AZT anabolites.

Female C57BL/6 mice at 20 weeks after inoculation with LP-BM5 murine leukemia virus, as well as age-matched control animals, were dosed subcutaneously with 25 mg/kg of AZT. The dosing solution contained [3H]AZT with a specific activity of 87 mCi/mmol. The levels of AZT and its phosphorylated anabolites were determined in tissues collected at different times after AZT administration using an analytical method coupling an ion-pair HPLC separation procedure with radioactivity detection after the separation.

The tissue-to-plasma AZT ratios in control mice could be ranked in the following order: kidneys > muscle ≈ spleen ≈ liver ≈ heart ≈ lung > thymus > lymph nodes > brain. Similar rank order was observed in infected animals, with the exception that significantly higher AZT levels were found in the lymph nodes, where the tissue-to-plasma AZT ratios were higher than those in thymus tissues.

Tissue AZT 5'-monophosphate profiles tended to parallel the AZT profiles in most tissues examined. Delays in the appearance of AZT 5'-diphosphate and AZT-TP were observed in all tissues tested. AZT-TP content was not detectable in any of the brain samples analyzed. The conversion of AZT to AZT anabolites was found to be highest in the spleen and bone marrow samples from both control and infected animals. Lymph nodes of the control animals showed poor ability to phosphorylize AZT to its active triphosphate moiety. This ability was significantly enhanced in infected animals.

We concluded that the in vivo disposition of AZT anabolites after a single dose administration of AZT is tissue-specific in mice and that experimentally induced chronic retrovirus infection resulted in the most significant changes in the distribution of AZT into the lymph nodes and in the phosphorylation of AZT in this important target tissue. Further studies are needed to relate the tissue-specific disposition of AZT anabolites to the therapeutic problems encountered with AZT treatment.

At present, AZT1 (zidovudine) remains the drug of choice for initiating AIDS therapy. Other schedules, including dideoxycytidine and dideoxyinosine alone or in combination with AZT and the recently approved combination use of protease inhibitors and AZT seem promising. AZT in itself is not active; it needs to be converted intracellularly by a series of cellular kinases to its phosphorylated anabolites to exert cytotoxic or antiviral activities. The intracellular metabolism of AZT has been examined extensively in the in vitro cell lines or in human PBMCs (1–4). Figure 1 illustrates the intracellular anabolism of AZT. Once AZT enters the cells by passive diffusion, AZT-MP is first produced by thymidine kinase. Thymidylate kinase is responsible for the conversion of AZT-MP to AZT-TP. The enzyme that catalyzes the conversion of AZT-TP to AZT-TP is believed to be the nucleoside diphosphate kinase. These phosphorylated anabolites can be converted back to the parent drug. Pharmacologically, AZT-TP competes for binding to the viral reverse transcriptase with cellular dTTP, which is required for DNA synthesis (1, 5). This inhibits the rate of synthesis of viral DNA. In addition, AZT-TP can act as an alternative substrate for reverse transcriptase and be incorporated into the growing DNA chain, leading to termination of the elongation process because no 3’ to 5’ phosphodiester linkage can be formed (5).

Similarly, cytotoxicity is attributed to the inhibition of cellular DNA polymerases and termination of cellular DNA elongation by AZT-TP. Furthermore, accumulation of high concentrations of AZT-MP in host tissues has been suggested to inhibit the activity of thymidylate kinase, resulting in decreased formation of the dTTP needed for DNA synthesis (6). The presence of high concentrations of AZT-MP has also been shown to suppress the 3’ to 5’ exonuclease activity, resulting in impaired DNA repair (7). Both of these changes associated with accumulation of high concentrations of AZT-MP might consequently lead to cytotoxicity.

From the understanding of the cellular pharmacology of AZT, it can be derived that the pharmacokinetic variables that could affect the efficacy and toxicity of AZT include not only the absorption and elimination of the parent drug, but also encompass the distribution of the parent drug into the target cells or tissues, the ability of AZT to be

1 Abbreviations used are: AZT, 3'-azido-3'-deoxythymidine; AIDS, acquired immunodeficiency syndrome; PBMC, peripheral blood mononuclear cell; AZT-MP, 3'-azido-3'-deoxythymidine 5'-monophosphate; AZT-TP, 3'-azido-3'-deoxythymidine 5'-triphosphate; dTTP, thymidine 5'-triphosphate; HIV, human immunodeficiency virus; MuLV, murine leukemia virus; MAIDS, murine acquired immunodeficiency syndrome; AMT, 3'-amino-3'-deoxythymidine; GAZT, 3'-azido-3'-deoxy-5'-glucuronoyltymidine.

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phosphorylated, and the rate of dephosphorylation of AZT anabolites. Because of their polarity, the phosphorylated AZT anabolites, once formed intracellularly, cannot easily permeate the biological membrane. Conventional pharmacokinetic studies determining the drug and metabolite levels in circulating plasma will not allow us to monitor and predict concentrations of the active AZT anabolites. This deficiency has resulted in lack of understanding of the in vivo disposition of the active phosphorylated AZT anabolites and has contributed in part to failure to optimize the therapeutic use of AZT.

Recently, a number of investigators have been attempting to determine the phosphorylated AZT anabolite levels in PBMCs collected from HIV-infected individuals undergoing AZT therapy (8, 9). These studies contribute to improvement of our understanding of the in vivo time courses of AZT anabolites levels in HIV-positive patients, but they are often limited by the number of blood samples that can be collected from patients, and the sensitivity and specificity of the analytical procedures used. Furthermore, Pantaleo et al. (10) and Embretson et al. (11) have shown that, in the early stage of the disease, the lymph nodes in HIV-positive individuals harbor much more virus than blood cells. In addition, it has been shown that the virus also infects thymus epithelial cells, which are vital for normal development of T cells (10, 11). The virus trapped in the lymph nodes and other lymphoid tissues probably destroys T cells throughout the course of infection. However, it is not clear whether the in vivo time courses of AZT anabolite levels in PBMCs represent those levels in the lymphoid tissues that are critical to the progression of the disease.

In this research, we aimed to examine the in vivo disposition of AZT and AZT anabolites in various target tissues and to investigate the effect of chronic retrovirus infection on the tissue disposition of AZT anabolites. The study was performed in female C57BL/6 mice, the effect of chronic retrovirus infection on the tissue disposition of AZT and AZT anabolites in various target tissues and to investigate the lymphoid tissues that are critical to the progression of the disease.

Materials and Methods

Chemicals and Reagents. AZT and AZT-TP were purchased from Moravek Biochemicals, Inc. (Brea, CA) and were obtained from the AIDS Research and Reference Reagent Program, National Institute of Allergy and Infectious Diseases (Rockville, MD). AZT-MP was purchased from Moravek Chemicals and Reagents. [Methyl-3H]AZT was obtained from the AIDS Research and Reference Reagent Program. The radiolabeled AZT was purified with HPLC to a purity >99%. All other reagents were of HPLC grade or of the highest grade commercially available.

Animals. Female C57BL/6 mice at 3–5 weeks of age (14–16 g) were obtained from Simosen Laboratory, Inc. (Gilroy, CA). To minimize exogenous infection, mice were maintained in microisolator cages. Cages, bedding, food, and water were autoclaved before use, and all cage changes and animal handling was performed in laminar air fume hoods. Animals were allowed to acclimate to this standard environment for 2 weeks before receiving any treatment. After this period, animals assigned to the virus-infected group were injected intraperitoneally with 0.1 ml of LP-BM5 MuLV as described previously (12). Control groups consisted of uninfected age-matched animals. The LP-BM5 MuLV inoculum contains B-tropic ecotropic and mink cell focus-inducing MuLVs and a putative defective genome. Animals infected with this virus inoculum develop progressive lymphadenopathy, splenomegaly, polyclonal B cell activation, and deficiencies in both B and T cell responses to polyclonal and antigenic stimuli (13, 14). Advanced stages of this infection are associated with enhanced susceptibility to opportunistic infections and development of B cell lymphomas. The infected animals live 5–6 months before succumbing to disease or constriction of airway by enlarged lymphoid tissues (13, 14). Immunological changes observed in such animals have been referred to as retrovirus-induced MAIDS (15).

Animal Experimentation. Animals at 20 weeks after virus inoculation, as well as age-matched control animals, were used in the study. On the study day, animals were injected subcutaneously with 25 mg/kg of AZT between 8:30 and 10:00 am. The dosing solution was prepared by mixing nonradioactive AZT with [3H]AZT to a final concentration of 10 mg/ml in normal saline and a specific activity of 87 mCi/mmol. At 30, 60, or 90 min after dosing, groups of four control mice were killed, and at 30 or 90 min after AZT administration, groups of four LP-BM5 MuLV-infected mice were killed via a brief CO2 exposure followed by cervical dislocation. Blood was collected from the inferior vena cava and plasma was separated. Liver, kidneys, spleen, heart, lung, thymus, major lymph nodes (axillary, superficial cervical, facial, inguinal, superior mesenteric, and iliac lymph nodes), brain and a piece of thigh muscle were collected and weighed in the order described. Saline flush of the cavities of femur and tibia was obtained for use as bone marrow samples, and the cell numbers in these samples were counted with a hemocytometer. Removal of all tissues was completed within 12 min. Due to cessation of blood flow, drug distribution among tissues during tissue removal should have been minimal. The tissues were homogenized immediately in 10 volumes of 5% (w/v) ice-cold trichloroacetic acid. The supernatant was collected after the homogenate had been centrifuged at 10,000g for 15 min at 4°C. The acid was immediately removed by extracting the supernatant with an equal volume of 20% tri-n-octylamine in pentane. The neutralized aqueous fraction was collected and evaporated to dryness under vacuum (Savant Speed-Vac). Dried residues were stored at −20°C until analyzed. Preliminary studies had been performed to examine the extraction of AZT and its anabolites from tissues by spiking the tissues with the authentic standard mixture. The extraction recovery of AZT and AZT anabolites was found to be >91%. Minimal degradation of AZT and AZT anabolites was found when the dried tissue samples were stored at −20°C for up to 4 months.

Analytical Methods. Separation of AZT and its anabolites was performed on a C18 column (Novapak, Waters Associates, Milford, MA) with a mobile phase composed of 0.2 M phosphate buffer containing 4 mM tetrabutylammonium hydrogen sulfate (pH 7.5) and acetonitrile in the ratio of 97.5:2.5 (v/v). The mobile phase was pumped at a flow rate of 1.5 ml/min with a Waters 501 pump. The UV absorbance was monitored at 270 nm with a Waters variable wavelength UV detector. The HPLC condition described herein allowed the separation of AZT from its phosphorylated anabolites and the
concomitant determination of two major hepatic metabolites of AZT, AMT and GAZT. A typical nonisotopic chromatograph is presented in fig. 2a. The retention times of AMT, AZT-MP, GAZT, AZT-DP, AZT, and AZT-TP were found to be 1.2, 6.2, 7.5, 9.8, 11.5, and 15.2 min, respectively.

On the assay day, dried tissue extracts were reconstituted with 150–400 μl of water containing nonradiolabeled AZT and AZT-TP, and an aliquot was injected onto the HPLC by an autoinjector (Waters WISP 717). The UV absorbance of the spiked nonradiolabeled AZT and AZT-TP in the samples was monitored to allow the proper assignment of retention time to each component. The effluent from the UV detector was fraction-collected every 20 sec (Dynamax FC-1 Fraction Collector, Rainin Instrument Co., Inc., Woburn, MA). Each effluent fraction was mixed with 6 ml of scintillation cocktail (UniverSol, ICN Biochemicals, Irvine, CA) and counted in a scintillation counter (Beckman LS 6800, Laguna Hills, CA). Radiolabeled AZT standards were injected daily, fraction-collected, and counted to ensure the consistency of recovery from the HPLC column. During the period of our analysis, the coefficient of variation of the radioactivity counts associated with the injected AZT standards was <7%. Representative radiochromatograms obtained after the injection of lymph node samples are shown in fig. 2, b and c.

Data Analysis. The absolute values of the plasma AZT or AZT metabolite levels were converted to molar concentrations based on the specific activity of the radiolabeled AZT. The tissue concentrations of AZT or AZT anabolites were presented as dpm per gram of tissue for most tissues examined and as dpm per 10¹⁰ cells for bone marrow samples. The ratios of tissue-to-plasma AZT levels and the ratios of tissue-to-plasma AZT-MP levels were estimated from the quotient of the tissue drug levels to the plasma levels of the respective compounds. The ratios of tissue concentrations of AZT anabolites to those of AZT were estimated to correct for differences in the extent of AZT tissue distribution.

A two-way analysis of variance was used to compare the tissue-to-plasma ratios of AZT between different treatments (i.e. between study groups or among different sampling times after dosing). In this analysis, study group and sampling time were included as the main effects, and the potential interaction between these effects was also examined. A p value < 0.05 for the main effects was considered to be statistically significant. However, when the interaction term was found to have a p value of <0.1, the following statistical tests were performed to examine the differences between different treatments. To examine the differences among different sampling times after dosing in control animals, data were analyzed with a one-way analysis of variance followed by Tukey’s test. An unpaired t test was used for the statistical comparisons of data collected at the two sampling times in infected animals. An unpaired t test was also used for comparisons of the estimates collected at the same sampling time of control and infected animals.

The statistical comparisons of the tissue-to-plasma ratios of AZT and AZT-MP determined at 90 min after dosing were performed using the analysis of variance for repeated measurements. In this analysis, the study group was treated as the main effect and the chemical form (parent drug or metabolite) was considered the repeated measurements. The potential interaction between the study group and the chemical form was also examined. A p value < 0.05 for the main effect or for the repeated measurement was considered statistically significant. However, when the interaction term was found to have a p value <0.1, an unpaired t test was used for comparisons of the estimates between treatments (i.e. between different study groups or between AZT and AZT-MP).

Results

The time courses of the plasma levels of AZT and AZT metabolites are shown in fig. 3. The plasma AZT concentrations declined from ~45 μM at 30 min after the subcutaneous administration of a 25 mg/kg dose of AZT to ~1 μM at 90 min after dosing. Comparable plasma AZT levels were found in control and infected animals. Interestingly, AZT-MP could be quantified in all the plasma samples collected from both control and infected animals. Its presence in plasma was confirmed by MS analysis (data not shown). AZT-DP and AZT-TP were not detectable in the plasma samples. Minimal differences in AMT, GAZT, or AZT-MP levels were observed between control and infected mice.

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![Fig. 2. Representative chromatograms.](https://example.com/fig2.png)

(a) A typical chromatogram obtained after injecting a mixture of nonradiolabeled standards. (b) A representative radiochromatogram obtained after the injection of a processed lymph node sample collected from control animals at 30 min after dosing. (c) A representative radiochromatogram obtained after the injection of a processed lymph node sample collected from LP-BM5 inoculated animals at 30 min after dosing.
Table 1 summarizes the tissue-to-plasma ratios of AZT for control and infected animals at different times after dosing. The tissue-to-plasma AZT ratios (g tissue/ml plasma) in control mice could be ranked in the following order: kidneys > muscle > spleen > liver > heart > lung > thymus > lymph nodes > brain. The extent of AZT distribution into bone marrow was difficult to compare with that into other tissues, because we were uncertain how many bone marrow cells would be equivalent to 1 g of tissue weight. Similar rank order was observed in infected animals, with the exception that significantly higher AZT levels were found in the lymph nodes, where the tissue-to-plasma AZT ratios in lymph nodes were higher than those in thymus tissues. As in control animals, the brain-to-plasma AZT ratios

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Bone Marrow</th>
<th>Lymph Nodes</th>
<th>Spleen</th>
<th>Thymus</th>
<th>Brain</th>
<th>Liver</th>
<th>Kidneys</th>
<th>Lung</th>
<th>Heart</th>
<th>Muscle</th>
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<tbody>
<tr>
<td>Control</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>30 min</td>
<td>0.698 ± 0.010</td>
<td>0.241 ± 0.010</td>
<td>0.745 ± 0.010</td>
<td>0.434 ± 0.010</td>
<td>0.062 ± 0.010</td>
<td>0.556 ± 0.010</td>
<td>2.616 ± 0.010</td>
<td>0.559 ± 0.010</td>
<td>0.588 ± 0.010</td>
<td>0.686 ± 0.010</td>
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<td></td>
<td>0.850 ± 0.265</td>
<td>0.379 ± 0.077</td>
<td>0.627 ± 0.800</td>
<td>0.493 ± 0.164</td>
<td>0.096 ± 0.007</td>
<td>0.568 ± 0.008</td>
<td>2.749 ± 0.008</td>
<td>0.633 ± 0.114</td>
<td>0.659 ± 0.115</td>
<td>0.769 ± 0.114</td>
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<tr>
<td>90 min</td>
<td>1.44 ± 0.161</td>
<td>0.418 ± 0.057</td>
<td>0.627 ± 0.058</td>
<td>0.405 ± 0.040</td>
<td>0.167 ± 0.017</td>
<td>0.518 ± 0.012</td>
<td>2.853 ± 0.045</td>
<td>0.655 ± 0.062</td>
<td>0.686 ± 0.048</td>
<td>0.876 ± 0.015</td>
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<td></td>
<td>0.766 ± 0.298</td>
<td>0.759 ± 0.145</td>
<td>0.755 ± 0.114</td>
<td>0.489 ± 0.017</td>
<td>0.071 ± 0.012</td>
<td>0.628 ± 0.014</td>
<td>1.64 ± 0.074</td>
<td>0.606 ± 0.095</td>
<td>0.648 ± 0.142</td>
<td>0.693 ± 0.142</td>
</tr>
<tr>
<td></td>
<td>0.261 ± 0.171</td>
<td>0.738 ± 0.018</td>
<td>0.677 ± 0.049</td>
<td>0.634 ± 0.097</td>
<td>0.642 ± 0.087</td>
<td>1.894 ± 0.087</td>
<td>1.207 ± 0.023</td>
<td>1.207 ± 0.087</td>
<td>0.701 ± 0.023</td>
<td>1.274 ± 0.023</td>
</tr>
</tbody>
</table>

a Tissue-to-plasma ratios are presented as dpm/10^10 cells over dpm/ml plasma.
b Tissue-to-plasma ratios are presented as dpm/g tissue over dpm/ml plasma.
c Significantly different from the corresponding values in the same study group at 30 min after dosing, p < 0.05.
d Significantly different from the corresponding values in the same study group at 60 min after dosing, p < 0.05.
e Significantly different from that determined at the respective times after dosing of the control animals, p < 0.05.

Table 1 summarizes the tissue-to-plasma ratios of AZT for control and infected animals at different times after dosing. The tissue-to-plasma AZT ratios (g tissue/ml plasma) in control mice could be ranked in the following order: kidneys > muscle > spleen > liver > heart > lung > thymus > lymph nodes > brain. The extent of AZT distribution into bone marrow was difficult to compare with that into other tissues, because we were uncertain how many bone marrow cells would be equivalent to 1 g of tissue weight. Similar rank order was observed in infected animals, with the exception that significantly higher AZT levels were found in the lymph nodes, where the tissue-to-plasma AZT ratios in lymph nodes were higher than those in thymus tissues. As in control animals, the brain-to-plasma AZT ratios
of infected animals were much lower than those in other tissues. Significantly higher lymph nodes-to-plasma AZT ratios were observed in the infected animals when these ratios were compared with those in controls (0.759 ± 0.145 vs. 0.241 ± 0.10, p < 0.0055, and 0.738 ± 0.018 vs. 0.418 ± 0.057, p < 0.05, at 30 and 90 min post-AZT administration, respectively). Lower tissue-to-plasma AZT ratios were observed in kidneys of infected animals than in those of controls. At 90 min after AZT administration, brain-to-plasma ratios of AZT of infected animals were lower than those of control animals.

The tissue-to-plasma AZT ratios in most tissues examined were found not to be dependent on the times of sampling (table 1), thus suggesting that the distribution of AZT into these tissues had attained equilibrium with the peripheral plasma by 30 min post-AZT administration. The tissue-to-plasma AZT ratios of the lymph nodes, bone marrow, and brain of control animals were significantly (p < 0.05) higher at 90 min after dosing than at 30 min after AZT administration (table 1). Delays in attaining distributional equilibrium of AZT between these tissues and peripheral plasma might have been responsible for the time-dependent differences in tissue-to-plasma ratios.

Table 2 summarizes the tissue-to-plasma ratios of AZT-MP for control and infected animals at 90 min after dosing. In control animals, the tissue-to-plasma ratios of AZT-MP of bone marrow, spleen, liver, and kidneys were significantly (p < 0.05) higher than those of AZT in the corresponding tissues. The most substantial changes in the tissue-to-plasma ratios after the addition of a phosphate group were observed in the spleen and bone marrow samples (from 0.627 ± 0.058 for AZT to 2.543 ± 0.907 for AZT-MP in the spleen and from 1.44 ± 0.161 for AZT to 6.31 ± 2.55 for AZT-MP in bone marrow). In infected animals, differences in the tissue-to-plasma ratios of AZT and AZT-MP were observed in various tissues. The most notable changes also were observed in the spleen samples (from 0.677 ± 0.049 for AZT to 2.08 ± 0.035 for AZT-MP). Differences between control and infected animals in tissue-to-plasma AZT-MP ratios were observed in a number of tissues, including lymph nodes, thymus, brain, kidney, and heart.

The tissue concentration-time profiles of AZT and AZT anabolites in spleen, lymph node, brain, muscle, bone marrow, and liver samples of control animals are shown in fig. 4. Tissue concentration-time profiles of AZT-MP tended to parallel the AZT profiles in these tissues and in other tissues examined. There were delays in the appearance of AZT-DP and AZT-TP in tissues shown and in most other tissues examined. AZT-TP content was not quantifiable in any of the brain samples analyzed. In the terminal phase, the declines of AZT-DP and AZT-TP levels in the liver seemed to parallel to those of the AZT and AZT-MP levels. Patterns similar to that observed in the liver were also observed in other nonlymphoid tissues, such as heart, kidney, and lung. The declines in AZT-TP levels as a function of time seemed to be slower in muscle, bone marrow, and the lymphoid tissues than such declines in AZT and AZT-MP levels.

The tissue concentration-time profiles of AZT and AZT anabolites in spleen, lymph node, brain, muscle, bone marrow, and liver samples of retrovirus inoculated animals are shown in fig. 5. For the limited time points collected, the overall profiles of AZT and AZT anabolites were comparable with those observed in the control animals. AZT-MP, AZT-DP, and AZT-TP levels in the pooled lymph nodes of the infected animals were significantly higher than the respective anabolites of the control animals (422,732 ± 237,454 vs. 92,887 ± 42,288 dpm/g tissue and 61,121 ± 18,455 vs. 6,728 ± 2,087 dpm/g tissue for AZT-MP at 30- and 90-min time points, respectively; 48,631 ± 34,373 vs. 2,374 ± 554 dpm/g tissue and 34,114 ± 5,955 dpm/g tissue vs. nonquantifiable levels for AZT-TP at 30- and 90-min time points, respectively, p < 0.05). However, AZT-DP levels in brain tissues of the control animals were found to be higher than those in infected animals (10,065 ± 1,892 vs. 5,385 ± 1,331 dpm/g tissue and 12,601 ± 5,739 vs. 6,079 ± 3,078 dpm/g tissue at 30- and 90-min time points, respectively, p < 0.05). No significant differences between control and infected animals in AZT anabolic levels were found in most other tissues examined.

The ratios of tissue levels of AZT-MP to AZT and of AZT-TP to AZT at 90 min after dosing are summarized in table 3. Data are presented in ratios to correct for differences in AZT distribution into different tissues and time-dependent changes in tissue levels of the precursor. Presenting data in ratios also reduces the interanimal variability resulting from differences in tissue distribution of AZT among animals. In addition, this presentation format allows the comparison of AZT anabolite data collected from bone marrow (absolute values were expressed in dpm/10^10 cells, whereas ratios were dimensionless values) with those in other tissues (absolute values were expressed in dpm/g tissue, whereas ratios were dimensionless values). Because of delays in the distribution of AZT into a number of tissues and in the appearance of AZT-TP in most tissues, only ratios at 90 min are presented and used as an approximate measurement for comparison of the extent of conversion of AZT to its anabolites. The highest ratios of AZT-MP to AZT were observed in the spleen and bone marrow samples. This suggests that AZT is more efficiently converted to AZT-MP in the spleen and bone marrow or that AZT-MP is preferentially accumulated in these tissues. The highest ratios of AZT-TP to AZT were also observed in the spleen and bone marrow samples. The AZT-TP to AZT ratios in an important lymphoid tissue, the thymus, were higher than those in other tissues examined. In control animals, AZT-TP levels were not quantifiable in the lymph nodes collected at 90 min after dosing. Very low AZT-TP/AZT ratios were observed in

### Table 2

Tissue-to-plasma ratios of AZT-MP at 90 min after dosing

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th>Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone Marrow</td>
<td>6.31</td>
<td>5.49</td>
</tr>
<tr>
<td>Lymph Nodes</td>
<td>0.490</td>
<td>1.16</td>
</tr>
<tr>
<td>Spleen</td>
<td>2.54</td>
<td>2.08</td>
</tr>
<tr>
<td>Thymus</td>
<td>0.451</td>
<td>1.11</td>
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<tr>
<td>Brain</td>
<td>0.148</td>
<td>0.057</td>
</tr>
<tr>
<td>Liver</td>
<td>1.38</td>
<td>1.074</td>
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<tr>
<td>Kidneys</td>
<td>4.32</td>
<td>2.23</td>
</tr>
<tr>
<td>Lung</td>
<td>0.813</td>
<td>0.892</td>
</tr>
<tr>
<td>Heart</td>
<td>1.13</td>
<td>0.60</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.781</td>
<td>0.478</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th>Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone Marrow</td>
<td>2.55</td>
<td>7.16</td>
</tr>
<tr>
<td>Lymph Nodes</td>
<td>0.124</td>
<td>0.314</td>
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<tr>
<td>Spleen</td>
<td>0.907</td>
<td>0.035</td>
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<td>Thymus</td>
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<td>0.274</td>
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<td>Brain</td>
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<td>0.019</td>
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<td>Liver</td>
<td>0.107</td>
<td>0.119</td>
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<tr>
<td>Kidneys</td>
<td>0.449</td>
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<td>Lung</td>
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<td>0.048</td>
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<tr>
<td>Heart</td>
<td>0.231</td>
<td>0.029</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.275</td>
<td>0.096</td>
</tr>
</tbody>
</table>

*a Tissue-to-plasma ratios are presented as dpm/10^10 cells over dpm/ml plasma.

*b Tissue-to-plasma ratios are presented as dpm/g tissue over dpm/ml plasma.

*c Significantly different from the respective tissue-to-plasma ratios of AZT at 90 min after dosing (AZT data are shown in table 1), p < 0.05.

*d Significantly different from the respective values of the control animals, p < 0.05.
the lymph nodes of control animals at 30 and 60 min after dosing (0.0012 ± 0.0004 and 0.0008 ± 0.0016 at 30 and 60 min after dosing, respectively). These ratios increased significantly in the infected animals (0.0094 ± 0.0068 and 0.071 ± 0.015 at 30 and 90 min after dosing, respectively).

**Discussion**

In this study, we demonstrated that the distribution of AZT and the disposition of AZT anabolites in mice after a single dose administration of AZT were tissue-specific and that chronic retrovirus infection
resulted in minimal changes in either the tissue distribution of AZT or the tissue disposition of AZT anabolites in animals with MAIDS. These results provide initial understanding of the in vivo tissue disposition of AZT and AZT anabolites and the effects thereon of chronic retrovirus infection, furnishing perspective on the possible contribution of tissue-specific formation and elimination of AZT anabolites to therapeutic problems associated with the use of AZT.

AZT-MP was detected in all the plasma samples analyzed in this study. To the best of our knowledge, the presence of AZT-MP in plasma samples had not been reported before our investigation. It has been suggested previously that cell membrane is partly permeable to AZT-MP (16). Failure to detect AZT-MP in plasma samples in other

Fig. 5. Tissue concentration-time profiles of AZT and AZT anabolites of LP-BM5 MuLV-inoculated animals.

Semilogarithmic plots of tissue levels of AZT and AZT anabolites of LP-BM5 MuLV-inoculated mice as a function of time after subcutaneous administration of a 25 mg/kg dose of AZT. The dosing solution contained [3H]AZT, with a specific activity of 87 mCi/mmol. Each point represents the average of four mice, and the cross-vertical bars represent ±1 SD of the mean.
phosphorylated AZT anabolites. The detection of AZT-MP in plasma published pharmacokinetic studies probably can be attributed to the use of analytical methods not designed for the quantification of the phosphorylated AZT anabolites. The detection of AZT-MP in plasma samples suggested that AZT-MP, once formed intracellularly, can be transported out of the cells and into the peripheral plasma. Because the presence of high concentrations of AZT-MP in host tissues has been suggested to contribute to AZT-associated cytotoxicity (6, 7), monitoring the plasma pharmacokinetics of AZT-MP rather than that of the parent drug via a sensitive analytic method (e.g., radioimmunoassay) could possibly provide information more relevant to predicting the toxicity of AZT treatment.

Furthermore, detection of cellularly formed AZT-MP in peripheral samples also suggests that AZT-MP could be transported into the cells or tissues from the peripheral plasma. Therefore, AZT-MP found in tissue samples could result from the intracellular phosphorylation of AZT within the tissue and/or distribution into the tissues from AZT-MP present in the circulating plasma. Figure 6 illustrates a modified intracellular anabolism scheme of AZT incorporating a tissue distribution process for AZT-MP. Based on our study design, it is difficult to differentiate the AZT-MP formed within a tissue from that distributed into the tissue from the AZT-MP formed in other tissues. Comparisons of the tissue-to-plasma ratios of AZT-MP (data in table 2) and of AZT (data in table 1) provided us with some interesting information. It was found that the tissue-to-plasma ratios of AZT-MP in bone marrow, spleen, liver, and kidney tissues of both control and infected animals were higher than the corresponding ratios of AZT, with the most significant changes observed in spleen and bone marrow. Consistently, the ratios of AZT-MP/AZT in these two tissues were found to be higher than those in other tissues (table 3). This suggests that AZT is more efficiently converted to AZT-MP in the spleen and bone marrow. Preferential accumulation of AZT-MP in the spleen and bone marrow could also be responsible for this observation.

Literature information on thymidine kinase, the catalyst responsible for the conversion of AZT to AZT-MP, tends to support our hypothesis that the formation of AZT-MP is tissue-specific. In eucaryotic cells, two different thymidine kinases have been found: thymidine kinase 1 and thymidine kinase 2 (17). High levels of thymidine kinase 1 activity have been found in proliferating and malignant cells, and a considerably lower level of thymidine kinase activity observed in resting cells has been attributed to the presence of thymidine kinase 2 activity, such as the spleen. Further studies are necessary to improve our understanding of the in vivo formation and distribution of AZT-MP.

In this study, AZT-DP and AZT-TP were not detectable in plasma samples but were found in significant amounts in a variety of tissue samples. This suggests that, because of an increase in polarity when additional phosphate groups are added onto the structure, AZT-DP and AZT-TP formed intracellularly will not diffuse or be transported out of the cells. Therefore, the presence of AZT-DP and AZT-TP in the tissue samples could be attributed to the subsequent phosphorylation of AZT-MP within also contribute to a fraction of AZT anabolites detected in the tissue samples. Based on our preliminary estimation, the latter possible source of AZT anabolites should account for only a minor fraction of the AZT anabolites found in the tissue samples.

The ratios of tissue levels of AZT-MP to AZT and of AZT-TP to AZT were estimated and the values at 90 min after AZT administration are presented in table 3. These ratios determined at one time point do not provide accurate determination of the extent of AZT conversion to the active or toxic moiety; however, they do furnish an initial approximation of such conversion. Differences in tissue disposition of AZT and AZT anabolites also became apparent when these ratios were compared among tissues. The highest ratios of conversion to AZT-MP and to the active triphosphate moiety were found in the spleen and bone marrow samples. This outcome could have resulted from more efficient conversion of AZT to the active or cytotoxic anabolites or preferential accumulation of these anabolites in these tissues. In combination with cell- or tissue-specific defense mecha-

### TABLE 3

Ratios of tissue levels of AZT phosphates to AZT at 90 min after dosing

<table>
<thead>
<tr>
<th>Bone Marrow</th>
<th>Lymph Nodes</th>
<th>Spleen</th>
<th>Thymus</th>
<th>Brain</th>
<th>Liver</th>
<th>Kidneys</th>
<th>Lung</th>
<th>Heart</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AZT-MP/AZT</td>
<td>0.269</td>
<td>0.073</td>
<td>0.266</td>
<td>0.067</td>
<td>0.055</td>
<td>0.179</td>
<td>0.096</td>
<td>0.081</td>
<td>0.102</td>
</tr>
<tr>
<td></td>
<td>±0.070</td>
<td>±0.010</td>
<td>±0.072</td>
<td>±0.045</td>
<td>±0.019</td>
<td>±0.008</td>
<td>±0.008</td>
<td>±0.011</td>
<td>±0.011</td>
</tr>
<tr>
<td>AZT-TP/AZT</td>
<td>0.239</td>
<td>0.0008*</td>
<td>0.152</td>
<td>0.038</td>
<td>NQ</td>
<td>0.0048</td>
<td>0.0010</td>
<td>0.0061</td>
<td>0.0013*</td>
</tr>
<tr>
<td></td>
<td>±0.099</td>
<td>±0.0016</td>
<td>±0.044</td>
<td>±0.060</td>
<td>±0.0031</td>
<td>±0.0002</td>
<td>±0.0031</td>
<td>±0.0011</td>
<td>±0.009</td>
</tr>
<tr>
<td><strong>Infected</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AZT-MP/AZT</td>
<td>0.246</td>
<td>0.122*</td>
<td>0.269</td>
<td>0.107</td>
<td>0.041</td>
<td>0.129</td>
<td>0.089</td>
<td>0.073</td>
<td>0.064*</td>
</tr>
<tr>
<td></td>
<td>±0.086</td>
<td>±0.018</td>
<td>±0.051</td>
<td>±0.055</td>
<td>±0.010</td>
<td>±0.032</td>
<td>±0.015</td>
<td>±0.014</td>
<td>±0.011</td>
</tr>
<tr>
<td>AZT-TP/AZT</td>
<td>0.109</td>
<td>0.071*</td>
<td>0.153</td>
<td>0.032</td>
<td>NQ</td>
<td>0.0071</td>
<td>0.0009</td>
<td>0.0038</td>
<td>0.0018*</td>
</tr>
<tr>
<td></td>
<td>±0.072</td>
<td>±0.015</td>
<td>±0.056</td>
<td>±0.022</td>
<td>±0.0022</td>
<td>±0.0006</td>
<td>±0.0026</td>
<td>±0.0014</td>
<td>±0.0072</td>
</tr>
</tbody>
</table>

*AZT-TP levels were not quantifiable in the samples collected at 90 min after dosing; data collected at 60 min after dosing are listed in the table for comparison.

NQ, AZT-TP levels were not quantifiable.

Significantly different from the respective values of the control animals, $p < 0.05$. 

(17). Tissue-specific thymidine kinase activity attributed to differences in the tissue distribution of these two isozymes has been previously reported. In research to determine thymidine kinase activity in several rat tissues (including liver, kidney, brain, and spleen), it was found that thymidine kinase activity in the spleen was significantly higher than that in other tissues (18). In addition, Arnèt et al., using tissue samples collected from human subjects who had undergone surgery, have shown that very low thymidine kinase activity was observed in brain and muscle samples, whereas >10 times greater activity was found in the infant thymus (19). These limited thymidine kinase activity studies in different tissues are consistent with our hypothesis that AZT could be more efficiently converted to the monophosphorylated anabolite in tissues with high thymidine kinase activity, such as the spleen. Further studies are necessary to improve our understanding of the in vivo formation and distribution of AZT-MP.
nism, the observed tissue-specific disposition of the cytotoxic AZT anabolites could contribute to the development of bone marrow toxicity after chronic AZT treatment. To confirm this possibility, examination of the tissue disposition of AZT anabolites after chronic AZT administration will be necessary.

The thymus, an important lymphoid tissue that provides the microenvironment necessary for the maturation of T cells, was found to have low AZT-MP to AZT ratios, but had AZT-TP to AZT ratios ~2–20 times those of other tissues. This suggests that the thymus may not possess high thymidine kinase activity, but can efficiently metabolize AZT-MP to the subsequent phosphorylated metabolites. The cells in the lymph nodes, one of the most important target cells/tissues in retrovirus infection, did not efficiently convert AZT to its active moiety in control animals, having AZT-TP/AZT ratios lower than those of most tissues examined (AZT-TP/AZT ratios were 0.0008 ± 0.0016 at 60 min after dosing and AZT-TP levels were not quantifiable at 90 min after dosing; table 3). However, these ratios at 90 min after dosing increased to 0.071 ± 0.015 (table 3) in the diseases animals. These changes are likely to be attributable to changes in the activity of the enzymes responsible for the addition of the third phosphate group, because when the ratios of AZT anabolites to their respective immediate precursors were estimated, only the ratios of AZT-TP/AZT-DP exhibited the same magnitude of change (data not shown).

AZT-TP content after a single dose administration of AZT was not quantifiable in any of the brain samples analyzed. This could be due to the presence of low concentrations of its precursors in the brain or to an inherent deficiency in the brain’s ability to phosphorylate AZT-DP to form AZT-TP. However, the absolute AZT-DP levels detected in the brain were higher than or comparable with those in a number of other tissues where quantifiable levels of AZT-TP could be found. This tends to suggest that the absence of quantifiable levels of AZT-TP in the brain after a single dose administration of AZT is not likely to have resulted from the presence of low concentrations of the immediate precursor of AZT-TP in the brain. At least two isoforms of nucleoside diphosphate kinase, the enzyme that catalyzes the conversion of AZT-DP to AZT-TP, have been shown to be present in a number of animal species, including mice (20), rats (21), and humans (20). Of the two isoforms (α and β) isolated from the rat, the α-isofrom, the major form detected in most rat tissues, varied in levels from one tissue to another, whereas the β-isofrom was detectable mainly in the brain and testes (22). Differences in the substrate specificity of these two isoforms were observed in the same study.

Therefore, the lack of detectable levels of AZT-TP in the brain samples could be attributed to AZT-DP’s being a poor substrate of the nucleoside diphosphate kinase isoform that is primarily located in the brain. If the same differences in substrate specificity and tissue distribution of nucleoside diphosphate kinase should be observed in humans, inadequate maintenance of AZT-TP levels in the brain could be responsible for failure to control neurological disorders associated with HIV infection after AZT treatment. Nevertheless, chronic AZT administration is likely to yield higher AZT levels in the brain. Whether an increase in the concentration of the parent drug would result in adequate maintenance of the levels of the active triphosphate form in the brain requires further attention.

In this study, progressive lymphadenopathy and splenomegaly were observed in animals inoculated with LP-BM5 MuLV. In infected animals, spleens frequently weighed 0.5 g or more and were filled with nodular masses of lymphoid cells. In addition, reductions in the number of white blood cells, erythrocytes, and platelets in the blood were observed in the inoculated animals (data not shown). In our earlier work with this disease model, deficiencies in both B and T cell responses to mitogenic stimuli were observed in the infected animals (23). These observed clinical manifestations in this animal model are similar to those described previously (24–26). In this in vivo disposition study, minor changes in the AZT anabolites to AZT ratios were observed in a number of tissues in the LP-BM5 MuLV infected animals. The most noteworthy change was observed in the lymph nodes. Significant increases in the distribution of AZT into the lymph nodes (an increase of ~2-fold) and in the conversion of AZT to the triphosphate form of AZT in this important target tissue (a 10- to more than 50-fold increase in AZT-TP/AZT ratios) were observed in the retrovirus inoculated animals.

Effects of chronic retrovirus infection on the phosphorylation of AZT and AZT anabolites have been examined in a limited number of studies. Furman et al. (1) have shown that the phosphorylation of AZT in proliferating H9 cells remained unchanged when cells were infected with HIV. In a different study, the in vitro AZT anabolism in PBMCs collected from five HIV-positive individuals was found to be similar to that obtained from HIV-negative persons (27). Because of the large inter- and intraindividual variations in the in vitro AZT anabolism, the authors concluded that more data are required to arrive at a definite conclusion about the difference between healthy and HIV-positive individuals with regard to AZT-phosphorylating capacity. Interestingly, in studies comparing the in vitro phosphorylation capacity between resting and mitogen-stimulated cells, the levels of
AZT nucleotides in resting PBMCs were ~60–150-fold lower than in mitogen-stimulated PBMCs (27), and ratios of AZT-TP/iTP in stimulated cells were found to be 10–17-fold higher than those in resting cells (28). In addition, in PBMCs collected after AZT administration, higher AZT anabolite levels were observed in AIDS patients on oral therapy of AZT for periods >4 months (9, 29) than in healthy individuals after having received only a single dose of AZT (9). Differences in the in vitro and in vivo observations could be explained by the presence of a more advanced stage of the disease in the in vivo studies or, alternatively, could have resulted from chronic AZT administration. Further studies are necessary to elucidate the mechanism(s) responsible for changes observed in MAIDS animals and to allow extrapolation of such findings to humans.

Hepatic metabolites of AZT, AMT, and GAZT, were detected in most plasma samples analyzed in this study. High levels of GAZT were found only in the liver samples, with minimal amounts present in other tissues (data not shown). Because of poor resolution of the radioactive AMT and the adjacent peaks in most tissue samples analyzed, we cannot report the tissue AMT levels with confidence. Because AMT has been implicated in AZT-associated bone marrow toxicity (30), further studies examining the tissue distribution of AMT and the disposition of its anabolites could help improve understanding of the role of AMT on AZT-associated tissue-specific pathology.

We conclude that the in vivo disposition of AZT anabolites in mice after single-dose administration of AZT is tissue-specific. Tissue-specific disposition of AZT anabolites may have resulted in adequate maintenance of AZT anabolite levels in specific target tissues, such as the lymph nodes and the brain, and resulted in high levels of AZT anabolites in the bone marrow samples after AZT treatment. Moreover, advanced-stage retrovirus infection in LP-BM5 MuLV-infected animals seemed to have minimal effects on the tissue distribution of AZT and tissue disposition of AZT anabolites in most of the tissues examined but to have resulted in significant changes in the AZT and AZT anabolite levels in the lymph nodes. Extrapolation of results obtained from control and retrovirus inoculated mice after single-dose administration of AZT to humans receiving chronic AZT treatment are complicated by factors such as species differences in the activities of cellular kinases, chronic AZT administration or concomitant use of other antiretrovirals in HIV-infected individuals, and different cellular responses to the mouse and human retroviruses. Further studies are needed to relate the tissue-specific disposition of AZT anabolites to the therapeutic problems encountered with AZT treatment.

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References


